

Title: CHIMERIC, HUMAN AND
HUMANIZED ANTI-GRANULOCYTE
ANTIBODIES AND METHODS OF USE
Inventor(s): GOLDENBERG et al.
Atty. Dkt. No.: 018733-1267

```

AGCATGTGATGACCCAGACTCCACTCTCCCTGCTGCTCAGTCTTGGAGATCAAGCTCCTCTTGCAGATCTAGTCAGAGCATGTGA
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TCGTAACACTACTGGGTGAGGTGAGAGGGACGGACAGTCAAGAACCTCTAGTTCCGAGGTAGAGAACGTCCTAGATCAGTCTCGTAACAT
1 27A B C
S I V M T Q T P L S L P V S L G D Q A S I S C R S S Q S I V 30
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CATAGTAATGAAACACCTATTTAGAAATGGTACCTGCAGAAACCCAGGCAGTCTCCAAACCTCCTCATCTACAAAGTTTCCAACCGATT
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
GTATCATTACCTTTGTGGATAAATCTTACCATGGACGCTTTGGTCCGTCAGAGGTTTGGAGGAGTAGATGTTTCAAAGGTTGGCTAAA
D E 30 40 50
H S N G N T Y L E W Y L Q K P G Q S P N L L I Y K V S N R F
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CDR1
TCTGGGTCCAGACAGTTTCACTGCAGTGGATCAGGGACAGATTTCACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
AGACCCAGGCTCTGTCCAAGTCAACGTCACCTAGTCCCTCTCTAAAGTGTGAGTTCTAGTCTCAGTCTCACCTCCGACTCTCTAGACCCCTCAA
60 70 80
S G V P D R F S G S G T D F T L K I S R V E A E D L G V
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TATTACTGCTTCAAGGTTACATGTTCTCCGACGTTCCGTTGGAGGACCAAGCTGGAAATCAAAACGGGTGATGCTGCACCAAGTGA
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
ATAATGACGAAAGTTCCRAAGTGTACAAGGAGGCTGCAAGCCACCTCCGTTGGTTCGACCTTTAGTTTGCCTGACTACGACGTGTTGACAT
90 100 108
Y Y C F Q G S H V P P T F F G G T K L E I K R
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CDR3
tccatcttccaccatccagtgaggatccggc
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
aggtagaagggtggtaggtcactcctagggcgg
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
339

```

Figure 1A shows the DNA sequence encoding MN3Vk cloned by RT-PCR and the predicted amino acid sequence. Underlined arrows indicate the PCR primer sequences. The putative CDR regions are in bold and underlined, and indicated. Nucleotide residues are numbered sequentially (right side). Kabat's Ig molecule numbering is used for amino acid residues (top of the residues).

```

CAGGTCCAACTGCAGGAGTCTGACCTGAGCTGAAGAAGCCCTGGAGAGACAGTCAAGATATCCTGCAAGGCTTCTGGGTATACCTTCAGA
90
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
GTCCAGGTTGACGTCCTCAGACCTGGACTCGACTTCTTTGGACCTCTCTGTCTAGTTCCTATAGGACGTTCCGAAGACCCCATATATGGAAGTCT
1
Q V Q L Q E S G P E L K K P G E T V K I S C K A S G Y T F R
10
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
AACTATGAATGAAGTGGGTGAACAGGCTCCAGGAAGGGTTAAAGTGGATGGGCTGGATAAACACCTACATCGGAGAGCCCAACATAT
180
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TTGATACCTTACTTGACCCACCTTTGTCGAGGTCCTTTCCCAATTTCCACCTACCCGACCTATTGTGATGTGACCTCTCGGTTGTATA
50
N Y G M N W V K Q A P G K G L K W M G W I N T Y T G E P T Y
40
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
GCTGATGACTTCAAGGACGGTTTGCCCTTCTCTTTGGAAACCTCTGCCAGACACTGCCTATTTCAGATCAACAACGTCAAAAATGAGGAC
270
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CGACTACTGAAGTCCCTGCCAAGAGAGAAACCTTTGGAGACGGTCTGGAGACGGTCTAGTGTTCGACGTTTTCCTCCTG
60
A D D F K G R F A F S L E T S A S T A Y L Q I N N V K N E D
70
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
ACGGCTACATATTCTGTGCAAGAAAGGATGGATGGATTTCACGGTAGTAGCCTCGACTACTGGGGCAAGGACCAAGGTCACCGTC
360
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TGCCGATGTATAAAGACACAGTTCCTTCCCTACCTACCTAAAGTTGCCATCATCGAGCTGATGACCCCGGTTCCCTGGTCCAGTGGCAG
90
T A T Y F C A R K G W M D F N G S S L D Y W G Q G T T V T V
100
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TCCTCA
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
AGGAGT
113
S S
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

```

Figure 1B shows the DNA sequence encoding MN3VH cloned by RT-PCR and the predicted amino acid sequence. Underlined arrows indicate the PCR primer sequences. The putative CDR regions are in bold and underlined, and indicated. Nucleotide residues are numbered sequentially (right side). Kabat's Ig molecule numbering is used for amino acid residues (top of the residues).

Title: CHIMERIC, HUMAN AND
HUMANIZED ANTI-GRANULOCYTE
ANTIBODIES AND METHODS OF USE
Inventor(s): GOLDENBERG et al.
Atty. Dkt. No.: 18733-1267

```

GACATCCAGCTGACCCAGACTCCACTCTCCCTGCCTGTCTAGTCTTGGAGATCAAGCTCCATCTCTTGCAGATCTAGTCAGACGATTGTA
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CTGTAGTCGACTGGGTCTGAGGTGAGAGGGACGACAGTCAGAACCTCTAGTTCCGAGGTAGAGAACGCTCTAGATCAGTCTCGTAAACAT
90
D I Q L T Q T P L S L P V S L G D Q A S I S C R S S Q S I V
30

CATAGTAATGGAACACCTATTTAGAAATGGTACCTGCAGAAACACGAGCCAGTCTCCAAACCTCCTCATCTACAAAGTTTCCAACCGATT
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
GTATCAATTACCTTTGTGGATAAATCTTACCATGGACGCTTTGGTCCGGTCAGAGGTTTGGAGGAGTAGATGTTTCAAAGGTTGGCTAAA
180

H S N G N T Y L E W Y L Q K P G Q S P N L L I Y K V S N R F
55
CDR1
TCTGGGGTCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
AGACCCAGGGTCTGTCCAAGTCACCGTCACCTAGTCCCTGTCTAAGTGTGAGTTCTAGTCTCACCTCCGACTCCTAGACCCCTCAA
270

S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V
85

TATTACTGCTTCAAGGTTCAATGTTCTCTCCGACGTTCCGTGGAGGCACCAAGCTGGAGATCAAAAGT
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
ATAATGACGAAAGTTCCAAGGTACAAGGAGGCTGCAAGCCACCTCCGTGGTTCGACCTCTAGTTTGCA
339

Y Y C F Q G S H V P P T F G G G T K L E I K R
108
CDR3
```

Figure 2A shows the DNA and amino acid sequences of cMN3Vk domain. The CDR regions are in bold, underlined, and indicated. Nucleotide residues are numbered sequentially. Kabat's Ig molecule numbering is used for amino acid residues (same as in Fig. 1A).

Title: CHIMERIC, HUMAN AND
HUMANIZED ANTI-GRANULOCYTE
ANTIBODIES AND METHODS OF USE
Inventor(s): GOLDENBERG et al.
Atty. Dkt. No.: 18733-1267

```
CAGGTCCAACCTGACGAGTCTGGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATATCTCTCAAGGCTTCTGGGTATACCTTCAGA 90
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
GTCCAGGTTGACGTCCTCAGACCTGACCTGACTTCTTGGACCTCTCTGTGAGTCTATAGGACGTTCCGAAGACCCATATATGGAAGTCT

Q V Q L Q E S G P E L K K P G E T V K I S C K A S G Y T F R 30

AACTATGAATGAACCTGGTGAAACAGGCTCCAGGAAGGTTTAAAGTGGATGGGTGATAAACAACCTACACTGGAGAGGCCAACATAT
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TTGATACCTTACTTGACCCACTTTTGCCGAGGTCCCTTCCCAATTTCACTACCCGACCTATTGTGGATGTGACCTCTCGGTTGTATA 180

N Y G M N W V K Q A P G K G L K W M G W I N T Y T G E P T Y 59
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CDR1

GCTGATGACTTCAAGGACGGTTTGCCCTTCTCTTTGGAAACCTCTGCCAGCACTGCCCTATTTCAGATCAACAACGTCAAAAATGAGGAC 270
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CGACTACTGAAGTTCCTTGCCAAACGGAAGAGAAACCTTTGGAGACGGTCGTGACGGATAAACGTCCTAGTTGTGACGTTTTTACTCCTG

A D D F K G R F A F S L E T S A S T A Y L Q I N N V K N E D 86

ACGGTACATATTTCTGTGCAAGAAAGGGATGGATGGATTTCACACGGTAGTAGCCTCAGCTACTGGGCGCAAGGACACCGTCCACCGTC
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TGCCGATGTATAAAGACACGTTCTTTCCCTACCTACCTAAAGTTGCCATCATCGGAGCTGATGACCCCGGTTCCCTGGTGGCCAGTGGCAG 360

T A T Y F C A R K G W M D F N G S S L D Y W G Q G T T V T V 111
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CDR3

TCCTCA
-----
AGGAGT

S S
366
113
```

Figure 2B shows the DNA and amino acid sequences of cMn3VH domain. The CDR regions are in bold, underlined, and indicated. Nucleotide residues are numbered sequentially. Kabat's Ig molecule numbering is used for amino acid residues (same as in Fig. 1B).

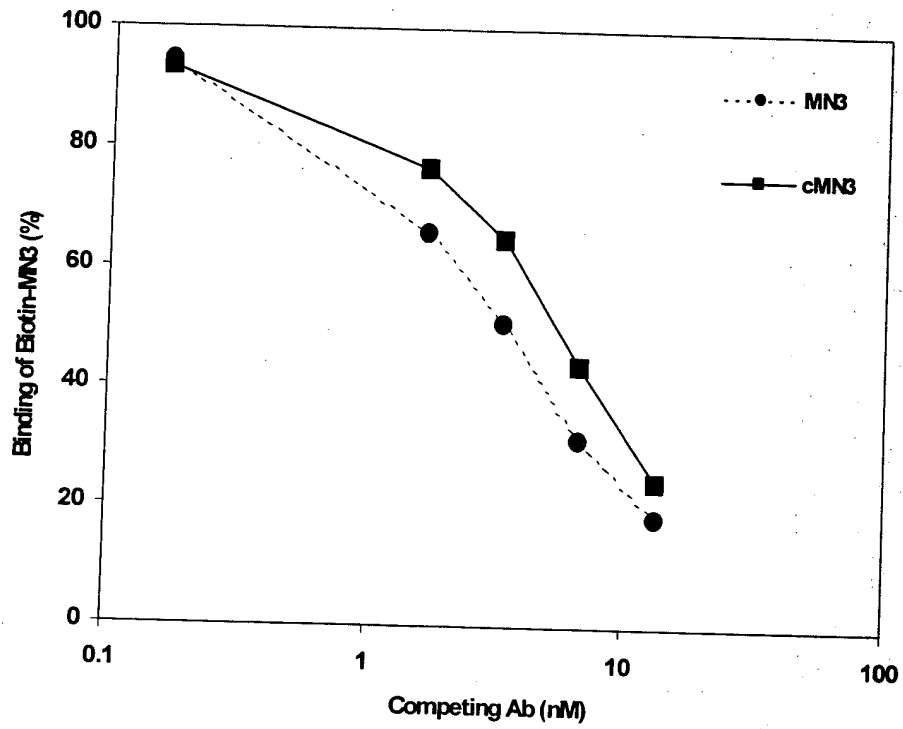


Figure 3.

Figure 4A. Amino acid sequence alignment of RE1, MN3 and hMN3 light chain variable domains. Dots indicate the residues in MN3 is identical to the corresponding residues in RE1. Dashes represent gaps introduced to aid the alignment. Boxed represent the CDR regions. Both N- and C-terminal residues (underlined) of hMN3 are fixed by the staging vector used. Therefore, the corresponding terminal residues of MN3 are not compared with that of RE1. Kabat's Ig molecule numbering scheme is used (same as in Fig. 1A).

EU_VH	1	10	20	30	40
MN3VH					
hMN3VH					
EU_VH		50	52 A	60	70
MN3VH					
hMN3VH					
EU_VH	80	82 A B C	90	100 A B C D E	110
MN3VH					
hMN3VH					
KOL_VH	103	110	113		
MN3VH					
hMN3VH					

PVQLVQSGAEVKKPGSSVKVCKASGGTFSRSAIIWVRQA
 QVQLQE•P•L•••ET•I••••Y••RNYGMN••K••
 QVQLQ••••••••••••••••Y••RNYGMN•••••
 PGQGLEWMGGIVPMFGPPNYAQKFQGRVTITADESTNTAY
 •K•K••W•NTYT•E•T•DD•K•FAFSLET•AS•••
 •••••W•NTYT•E•T•DD•K•FAF••••••••••
 MELSSLRSEDATFYFCAGGYGIYS-----PEEYNGGLVTV
 LQINNKN•••T•••RKGWMDENGSSLDY
 ••••••••••••••RKGWMDENGSSLDY
 WGQGTPTVTVSS
 ••••T•TVSS
 •••••TVSS

Figure4B. Amino acid sequence alignment of EU (FR1-3) and KOL (FR4), MN3 and hMN3 heavy chain variable domains. Dots indicate the residues in MN3 is identical to the corresponding residues in REI. Dashes represent gaps introduced to aid the alignment. Boxed represent the CDR regions. Both N- and C-terminal residues (underlined) of hMN3 are fixed by the staging vector used. Therefore, the corresponding terminal residues of MN3 are not compared with that of human VH sequences. Kabat's Ig molecule numbering scheme is used (same as in Fig. 1A).

XbaI
tctagacacaggaacctcaccatgggatggagctgtatcatcctcttcttggtagcaacagctacaggtat
M G W S C I I L F L V A T A T
aggggctcacagtagcaggcttgaggacatatatatatgggtgacaatgacatccactttgccttttctctccacAGGTGTCCACTCC
G V H S
PvuII
GACATCCAGCTGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTGGGTGACAGAGTGTCCATCTCTTGTAGATCCAGTCAGAGCATTTGTA 90
D I Q L T Q S P S L S A S V G D R V S I S C R S S Q S I V 27C
CATAGTAATGGAAACACCTATTAGATGGTACCAGAGAGCCAGGTAAGGCTCCAAAGCTGCTGATCTACAAAGTTTCCACCGATT 180
H S N G N T Y L E W Y Q Q K P G K A P K L L I Y K V S N R F 55
CDR1
TCCGGAGTCCAGACAGATTCAAGCGGTAGCGGTACCGACTTCACCTTCACGATCAGCAGCCTCCAGCAGAGGACATCGCCACC 270
S G V P D R F S G S G T D F T I S S L Q P E D I A T 85
BgIII/BclI
TACTACTGCTTTCAGGTTACATGTTCCCTCCGACGTTCGGCGGGGGACCAAGGTGGAGATCAAAACgtgagtagaatttaaaccttgct 337
Y Y C F Q G S H V P P T F G G G T K V E I K 107
CDR3
BamHI
tcctcagttggatcc

Figure 5A.

XhoI
ctcgaacacacaggaacctcaccATGGATGGAGCTGTATCATCTCTTCTTGGTAGCAACAGCTACagga
M G W S C I I L F L V A T A T

aggggtcacagtagcaggcttgaggcttgacatatatatgggtgacaatgacatccactttgccttttctctccacAGGTGTCCACTCC
G V H S

PstI
CAGGTCCAACTGCAGAGCTGTGGAGCTGAGGTCAAGAGCCCTGGATCTAGCGTCAAGGTCTCCTGCAAGGCTTCTGGGTATACCTTCAGA 90
Q V Q L Q Q S G A E V K K P G S S V K V S C K A S G Y T F R 30
AACTATGGAATGAACCTGGGTGAGACAGGCTCCAGGACAGGGTTTAGAGTGGGTGGATAAACACCTACACCGGTGAGCCCAACATAT 180
N Y G M N W V R Q A P G Q G L E W M G W I N T Y T G E P T Y 59
CDR1

CDR2
GCTGATGACTTCAAGGGAGCGTTTGCCTTCAAGCCGACGAATCTACCAACACTGCCTATATGGAGCTGTCTAGCTTGAATCTGAGGAC 270
A D D F K G R F A F T A D E S T N T A Y M E L S S L R S E D 75
ACGGCTTCTATTCTGTCAAGAAAGGGATGGATGGATTTCACCGGTAGTACCTCGACTACTGGGGCCAGGGACCCCGGTCCACCGTC 360
T A F Y F C A R K G W M D F N G S S L D Y W G Q G T P V T V 111
CDR3

BstEII
TCCTCAGgtgagtccttacacacctctctcttattcagcttaaatagattttactgcatttgttgggggaaatgtgtgtatctgaat 367
S S 113

BamHI
ttcaggtcatgaaggactagggaacaccttgggagtcagaaaaggtcatttgggagcccggtgatgcagacagacatcctcagctcccag
acttcagggccagagatttataggatccc

Figure 5B.

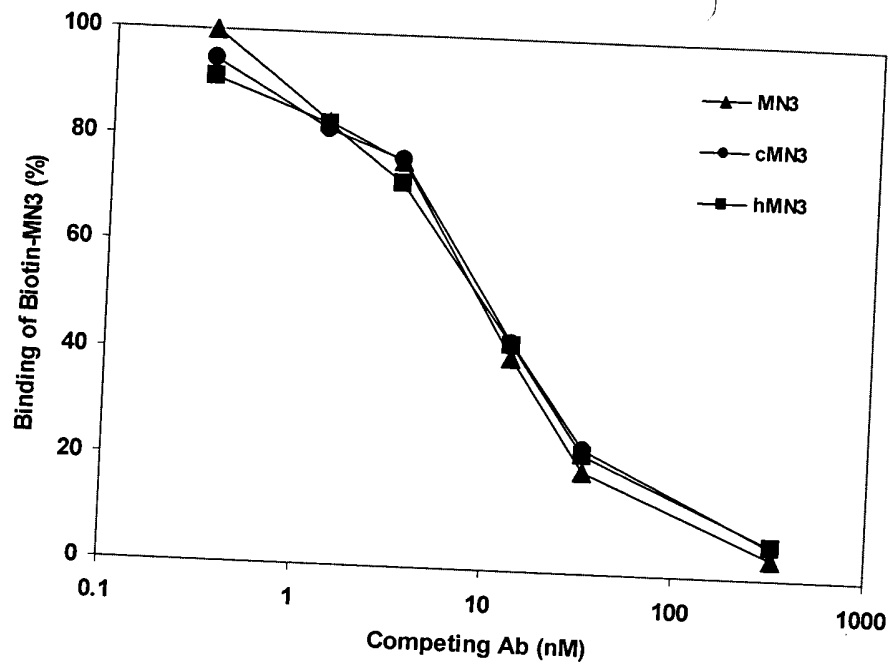


Figure 6.